DOI : 10.5958/0974-0112.2020.00005.5

Indian J. Hort. 77(1), March 2020: 65-71

Physiology of flowering in *Citrus* species

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ABSTRACT

An experiment was conducted on the trees of acid limes (Pusa Udit, Pusa Abhinav and ALC-29), lemons (Konkan Seedless, Kagzi Kalan and Hill lemon) and sweet oranges (Pusa Sharad, Pusa Round and Mosambi) during 2017-18 with the objectives to understand the relationship of seasonal changes in physio-biochemical traits with the flowering behaviour of citrus species. Sweet oranges and Hill lemon expressed flowering once in a year (February to March), while lemons and acid limes bloomed almost round the year. The flowering genotypes showed lower value of photosynthetic rate (*A*) than non-flowering genotypes. C:N ratio ≥ 5.57 could initiate the flowering in lemon (except Hill lemon) and limes, while in other genotypes, it was ≥ 13.39 for flowering. In sweet orange and Hill lemon, GA_3 followed an upward trend till January, and declined thereafter, however, the trend was reverse in other genotypes till December. During spring season, the trees having GA_3 ranging between 0.12-0.93 mg g^{-1} FW showed the flowering. The content of free amino acids (FAA) increased till February and declined thereafter. Overall the level of FAA ≥ 10 mg g^{-1} FW proved to be the critical level to induce flowering in all the genotypes tested. The level of total antioxidant capacity (TAC) ≥ 18.12 µmol g^{-1} FW favoured the flowering in all citrus genotypes tested. Overall low level (96.45-108.27 mmol H_2O_2 mg^{-1} FW) of hydrogen peroxide (H_2O_2) favoured the flowering.

Key words: C:N ratio, free amino acids, total antioxidant capacity, hydrogen peroxide.

INTRODUCTION

Flowering represents a phase transition from vegetative to reproductive growth. This phenomenon is one of the most important events in the plant life cycle because it is the initial stage in the sequence of producing a new generation. The change from vegetative to reproductive growth is called the flowering transition, which is controlled by inheritance, internal and external factors, and is accompanied by various biochemical, physiological, cytological and morphological changes in the bud leading to the formation of reproductive structures.

Citrus is a perennial tree crop, exhibiting a very peculiar and unusual reproductive physiology, showing a wide range of behaviours regarding both flowering time and response to the inductive conditions particularly in subtropical plains of northern India. Citrus trees usually have several flushes of growth during the year. The number of flushes and their importance are determined by cultivar characteristics, crop load and climate. For example, sweet orange, Kinnow and grapefruit tend to show flowering once in a year rigidly during spring season responding to cold inductive temperature. Lemon flower twice in a year (spring and late summer seasons), while lime trees show sparse flowering round the year in sub-tropical conditions, and exhibit higher floral responses to water stress than to cold inductive temperatures (Chaikiatiyyos *et al*., 3). Flower formation occurs in response to chilling temperatures, and the number of flowers formed can increase with the duration of exposure to low temperature, and it has two separate effects, as it releases bud dormancy and induces flowering.

Various biochemical constituents and phytoharmones have been reported to control flowering. Carbohydrates, protein and amino acids are important for flowering, but their relationship with flowering still not fully understood. The inhibitory effect of GA_3 on flowering by exogenous application in citrus has been well documented, also at the anatomical level (Lord and Ekard, 13), but the endogenous effect is still unknown. Antioxidants are compounds produced by aerobic organisms to counteract oxidative stress caused by an imbalance of reactive oxygen species (ROS). ROS include the superoxide radicals and the hydroxyl radicals produced as by-products of oxidation/reduction (redox) reactions as a consequence of aerobic metabolism. The examination of the antioxidant capacity of flowers was mainly focused on annuals flowers, while few studies were conducted on the flowers of woody fruit trees to evaluate the efficiency of scavenging free radicals (Kaur *et al*., 7).

Poor understanding about the biochemical and molecular mechanisms, involved in the regulation of flowering is the major constraint in the improvement

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of floral limit and frequency, and ultimately the productivity of fruit crops. Despite much research, citrus flowering remains poorly understood with many contradictions and conflicts existing between reports. Thus, the experiment was carried out to study the seasonal changes in the physio-biochemical aspects and their relationship with flowering in the citrus species of diverse nature.

MATERIALS AND METHODS

The present study was conducted in the Experimental Orchard of the Division of Fruits and Horticultural Technology, I.A.R.I., New Delhi during August, 2017 to March, 2018 on three *Citrus* species with three genotypes of each (Table 1). Four shoots on each plant were selected and tagged, and the data on various physio-biochemical parameters were recorded at monthly interval. The experiment was laid out in Randomized Block Design and replicated thrice. The flowering status of the trees of selected citrus species in terms of just appearance of white flowering tip was expressed as 'Yes', and 'No'.

The photosynthetic rate (*A*) was measured on four mature leaves on each replication using LCi-SDUltra Compact Photosynthesis System (ADC BioScientific Ltd., Global House, Hoddesdon, UK) from 2nd week of August, 2017 to 2nd week of March, 2018 and expressed in μmol $m^{-2}s^{-1}$. Measurements were performed between 11.00 and 1.00 P.M., and data were transferred to computer for analysis.

Four months-old-mature 30 leaves were collected from each replication (tree) for determination of leaf N. The digestion of leaf sample for the estimation of nitrogen was carried out in concentrated H_2SO_4 in the presence of a digestion mixture. The total leaf nitrogen content was estimated by Kjeldahl method (Kirk, 8). The total carbohydrate content was estimated using anthrone reagent (Hedge and Hofreiter, 6). The C:N ratio was calculated by dividing total leaf carbohydrate content with leaf nitrogen content. Gibberellic acid (GA_3) content was estimated from young leaves by using high performance liquid chromatography (HPLC) as per the method of Gaskin *et al.* (5). The HPLC conditions in this study were mobile phase composition: acetonitrile:water (70:30), flow rate (0.5ml/min), run time (10 min) and column: RPC_{18} (25CM, 0.25MM, 4μM) and wave length: 206 nm.

Table 1. *Citrus* species used in the study.

S. No.	Species	Genotypes
1.	*C. limon* (L.) Burm	Kagzi Kalan, Konkan Seedless and Hill lemon
2.	*C. aurantifolia* (Christm.) Swingle	Pusa Udit, Pusa Abhinav and ALC-29
3.	*C. sinensis* (L.) Osbeck	Mosambi, Pusa Round and Pusa Sharad

Cleaned leaf sample of 0.2 g was homogenized in mortar and pestle by adding 2.0 ml of 80% ethanol. The homogenate was collected in oak-ridge tubes and centrifuged at 15000 × g for 20 min at 4°C (Model-HERMEL Z 323K). The supernatant so obtained was stored in refrigerator to be used as extract for the estimation of total antioxidant capacity and free amino acids. The activity of free amino acids in leaf sample was determined by the method proposed by Moore and Stein (14). The total antioxidant capacity was assayed according to the method described by Wayner *et al*. (19). Hydrogen peroxide from four-month-old fully expanded leaves was estimated by forming titanium-hydro peroxide complex (Rao *et al.*, 15).

The data were analysed statistically using two-way analysis of variance, followed by Tukey's Honest Significant Difference (HSD) test available in SAS Software Version 9.3 (SAS Institute, Cary, NC, USA). *P* values ≤ 0.05 were considered significant.

RESULTS AND DISCUSSION

Results revealed that only ALC-29 lime and Kagzi Kalan lemon behaved as everbearer throughout the period of sampling. Pusa Abhinav lime and Konkan Seedless lemon flowered from September to February, while except August and October, Pusa Udit lime maintained the flowering status during the remaining months of sampling. In the months of February and March, all the genotypes tested expressed the flowering (Table 2).

Various citrus genotypes were found to differ significantly in respect of *A* measured at monthly intervals between August to March (Table 3). In general, highest value of *A* was noticed in August, thereafter followed a downward trend till December, which increased gradually towards the approach of spring season. In the month of August, highest *A* (12 μmol m^{-2} s^{-1}) was noticed with Hill lemon having similarity statistically with Konkan Seedless lemon and Pusa Abhinav lime. Hill lemon tended to show the highest *A* during September, November, December and January. In the month of February and March, all lemon and sweet orange genotypes were found statistically similar in respect of *A*. It is clear from the data that in each month of data recording, flowering genotypes showed lower *A* than non-flowering genotypes. Shivshankara and Mathai (16) reported significantly low net photosynthetic rate in flowering branches, whereas higher net photosynthetic rate

Table 2. Flowering status of citrus genotypes during August 2017 to March 2018.

Species	Genotype	Month							
		Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar
C. aurantifolia	Pusa Udit	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Pusa Abhinav	No	Yes	No	Yes	Yes	Yes	Yes	Yes
	ALC-29	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
C. limon	Konkan Seedless	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Kagzi Kalan	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Hill Lemon	No	No	No	No	No	No	Yes	Yes
C. sinensis	Pusa Sharad	No	No	No	No	No	No	Yes	Yes
	Pusa Round	No	No	No	No	No	No	Yes	Yes
	Mosambi	No	No	No	No	No	No	Yes	Yes

Table 3. Seasonal variation in the photosynthetic rate (*A*) (µmol m^{-2} s^{-1}) of citrus genotypes.

Species	Genotype	Month							
		Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar
C. aurantifolia	Pusa Udit	11.12^{b}	9.02^{fe}	7.10^{d}	5.12^{c}	3.13^{fe}	3.14^{e}	4.10^{c}	4.25^{b}
	Pusa Abhinav	11.55^{ba}	9.23^{fde}	7.56^{a}	5.14^{c}	3.20^{de}	3.22^{e}	4.18^{bc}	4.29^{ba}
	ALC-29	10.05^{c}	8.88^{f}	7.02^{d}	5.09^{c}	3.05^{f}	3.10^{e}	4.07^{c}	4.22^{b}
C. limon	Konkan Seedless	11.86^{a}	10.17^{b}	7.42^{bac}	5.33^{cb}	3.67^{b}	3.70^{b}	4.32^{ba}	4.45^{ba}
	Kagzi Kalan	10.18^{c}	9.84^{cb}	7.23^{bdc}	5.46^{b}	3.57^{b}	3.69^{b}	4.30^{ba}	4.39^{ba}
	Hill Lemon	12.00^{a}	10.85a	7.48^{ba}	5.96^{a}	3.95^{a}	3.98^{a}	4.36^{a}	4.50^{a}
C. sinensis	Pusa Sharad	10.27^{c}	9.57^{cd}	7.28^{bdac}	5.33^{cb}	3.57^{b}	3.59^{cb}	4.40^{a}	4.52^{a}
	Pusa Round	10.05^{c}	9.45^{cde}	7.20^{bdc}	5.27^{cb}	3.41^{c}	3.48^{cd}	4.36^{a}	4.45^{ba}
	Mosambi	9.78^{c}	9.18^{fde}	7.15^{dc}	5.22^{cb}	3.32^{dc}	3.38^{d}	4.33^{ba}	4.37^{ba}
LSD ($P \leq .05$)		0.51	0.48	0.30	0.30	0.13	0.14	0.16	0.23

Values are means (n=3). Mean values in each citrus cultivar followed by different lower-case letters were significantly different at ≤ *0.05* by Tukey's HSD test.

was reported in the non-flowering branches of mango tree. Leaves close to inflorescence had lower rates of mitochondrial respiration and net photosynthesis and lower stomatal conductance and quantum efficiency of photosystem II under actinic light than vegetative shoot leaves (Urban *et al*., 17).

The C:N ratio showed the significant difference among all the citrus genotypes calculated at various dates of sampling (Table 4). The lowest C:N ratio was recorded in the month of August, which rose gradually till October, thereafter it increased rapidly registering its highest value in the month of February, and declined thereafter in all the genotypes tested. In limes and lemons (except Hill lemon), C:N ratio ≥ 5.57 could initiate the flowering, while in rest of the genotypes, it favoured the flowering after reaching at ≥ 13.39 indicating that the everbearer type citrus fruits respond to flowering at lower C:N ratio than those fruits when bloom once in a year. This is well supported from the fact that a high endogenous ratio of carbon to nitrogen in plants is stimulatory to flowering whereas a low C:N ratio favours vegetative growth (Corbesier *et al*., 4). High C:N ratio favours flower formation, and excessive N fertilization inhibits it. Petals also store carbohydrates that serve an important function during flower opening. For this, reserve polysaccharides (starch and/or fructans) are accumulated gradually during petal development but degraded rapidly at the onset of anthesis to generate the osmotic potential that leads to cellular water influx and, finally, to flower opening (Van Doorn and Kamdee, 18).

The level of GA_3 in the buds and young shoots of citrus genotypes expressed no significant variation, but their fluctuation during the course of present experimentation was variety specific (Table 5). In Pusa Sharad and Hill lemon, GA_3 followed an upward trend till January, and declined sharply during February. In

Table 4. Seasonal variation in the C/N ratio of citrus genotypes.

Species	Genotype	Month							
		Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar
C. aurantifolia	Pusa Udit	4.79^{c}	6.08^{bc}	6.68^{b}	7.25^{c}	10.39^{bc}	12.60^{d}	14.20^{b}	11.47^{b}
	Pusa Abhinav	4.75^{dc}	6.18^{ba}	5.39^{d}	7.28^{c}	11.15^{a}	14.25^{b}	14.97^{a}	10.96^{cb}
	ALC-29	6.52^{a}	6.51^{a}	6.76^{ba}	7.76^{b}	9.83^{c}	14.90^{a}	15.29^{a}	12.80^{a}
C. limon	Konkan Seedless	4.64^{dc}	5.75^{c}	5.98^{c}	7.95^{b}	10.79^{ba}	13.65^{c}	14.15^{b}	12.81^{a}
	Kagzi Kalan	5.57^{b}	6.44^{ba}	7.22^{a}	9.53^{a}	10.04^{c}	12.92^{d}	14.02^{cb}	12.51^{a}
	Hill Lemon	4.57^{dc}	4.25^{d}	4.81^{e}	6.79^{d}	8.69^{d}	13.81^{cb}	13.65^{cb}	10.99^{cb}
C. sinensis	Pusa Sharad	4.56^{dc}	4.27^{d}	4.91^{e}	6.07^{e}	8.31^{ed}	10.56^{e}	13.39^{c}	10.42^{cd}
	Pusa Round	4.52^{dc}	4.28^{d}	4.90^{e}	6.19^{e}	7.92^{e}	11.04^{e}	13.55^{cb}	10.22^{d}
	Mosambi	4.43^{d}	4.24^{d}	4.80^{e}	6.06^{e}	8.64^{d}	12.38^{d}	14.19b	10.92^{cbd}
LSD ($P \leq 0.05$)		0.32	0.37	0.47	0.39	0.69	0.55	0.70	0.73

Values are means (n=3). Mean values in each citrus cultivar followed by different lower-case letters were significantly different at $P \leq 0.05$ by Tukey's HSD test.

Table 5. Seasonal variation in the level of gibberellic acid (GA_3) (mg g^{-1} FW) of citrus genotypes.

Species	Genotype	Month							
		Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar
C. aurantifolia	Pusa Udit	1.05^{cd}	0.64^{g}	0.68^{e}	0.49^{e}	0.32^{f}	0.92^{c}	0.21^{c}	0.28^{a}
	Pusa Abhinav	1.13^{b}	0.74^{f}	0.87^{d}	0.43^{f}	0.36^{e}	0.93^{c}	0.13^{f}	0.25^{b}
	ALC-29	0.67^{f}	0.95^{e}	0.83^{d}	0.41^{f}	0.38^{e}	0.88^{c}	0.28^{b}	0.23^{c}
C. limon	Konkan Seedless	1.21^{a}	0.47^{h}	0.22^{f}	0.23^{h}	0.12^{g}	0.66^{e}	0.18^{d}	0.20^{d}
	Kagzi Kalan	0.57^{g}	0.42^{i}	0.25^{f}	0.30^{g}	0.13^{g}	0.75^{d}	0.22^{c}	0.24^{cb}
	Hill Lemon	1.23^{a}	1.23^{b}	1.21^{c}	1.18^{d}	1.10^{d}	1.52^{b}	0.15^{e}	0.18^{e}
C. sinensis	Pusa Sharad	1.01^{ed}	1.19^{c}	1.42^{a}	1.51^{a}	1.68^{a}	1.74^{a}	0.13^{f}	0.17^{e}
	Pusa Round	1.09^{cb}	1.35^{a}	1.37^{b}	1.47^{b}	1.62^{b}	1.72^{a}	0.32^{a}	0.27^{a}
	Mosambi	0.98^{e}	1.10^{d}	1.38^{a}	1.33^{c}	1.58^{c}	1.70^{a}	0.16^{e}	0.21^{d}
LSD ($P \leq 0.05$)		0.05	0.03	0.04	0.03	0.04	0.07	0.01	0.01

Values are means (n=3). Mean values in each citrus cultivar followed by different lower-case letters were significantly different at $P \leq 0.05$ by Tukey's HSD test.

rest of the genotypes, the reverse trend was noticed, and its highest level was recorded in January, which declined sharply in the month of February and increased minutely thereafter. In flowering trees of lemon and limes between August to January, the level of GA_3 ranged between 0.12-0.93 mg g^{-1} FW**.** During spring season (February to March), when all the genotypes behaved as floriferous, the overall GA_3 was quite low (0.13-0.32 mg g^{-1} FW). Gibberellic acid induced floral inhibitions have also been reported in citrus, whereas reduced levels of endogenous GA_3 have been correlated with floral initiation in citrus (Koshita *et al*., 9). GA_3 inhibit floral induction by initiation of a high proportion of vegetative shoots at the expense of reproductive shoots during floral-inductive conditions (Boss and Thomas, 1). It has also been reported, GA 2-oxidase genes encoding enzymes with GA inactivation activity, CuGA2ox2/3 and CuGA2ox8 were more highly expressed in flower buds of Satsuma mandarin (*Citrus unshiu* Marc.) (Kotoda *et al*., 10).

The (free amino acids) FAA content was found to differ significantly in tested genotypes estimated at various date of sampling (Table 6). The level of FAA in single bloom species (Hill lemon, and sweet oranges) recorded between 6.28-8.35 mg g^{-1} FW in August, and increased steadily till January, thereafter increased sharply, reaching at peak in February and declined towards the approach of March. The content of FAA was quite higher initially in Kagzi Kalan lemon (10.94 mg g^{-1} FW) and ALC-29 lime (11.37 mg g^{-1} FW) which increased gradually till January, and reached

Table 6. Seasonal variation in the free amino acid content (mg g^{-1} FW) of citrus genotypes.

Species	Genotype	Month							
		Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar
C. aurantifolia	Pusa Udit	4.23^{f}	11.38^{cb}	13.67^{ba}	13.64^{a}	12.58^{b}	13.72^{b}	14.76^{e}	10.62^{ed}
	Pusa Abhinav	6.21^{e}	11.21^{c}	7.31^{e}	12.92^{b}	13.82^{a}	13.08^{c}	14.47^{e}	10.17^{e}
	ALC-29	11.37^{a}	11.64^{b}	13.22^{bc}	13.97^{a}	13.10^{b}	14.10^{a}	15.05^{de}	10.95^{d}
C. limon	Konkan Seedless	6.12^{e}	12.23^{a}	13.16^{c}	13.68^{a}	14.02^{a}	14.30^{a}	16.13^{c}	12.02^{c}
	Kagzi Kalan	10.94^{b}	12.48^{a}	13.99^{a}	13.77^{a}	13.96^{a}	14.21^{a}	16.26^{bc}	12.1^{c}
	Hill Lemon	6.28^{e}	6.45^{f}	6.58^{f}	6.64^{d}	7.64^{d}	8.88^{e}	17.38^{a}	14.3^{a}
C. sinensis	Pusa Sharad	7.26^{d}	7.47^{e}	7.55^{e}	6.68^{d}	7.55^{d}	8.78^{e}	15.74^{dc}	12.54^{c}
	Pusa Round	8.35^{c}	8.58^{d}	8.67^{d}	8.71^{c}	9.05^{c}	9.30^{d}	16.86^{ba}	13.66^{b}
	Mosambi	8.18^{c}	8.40^{d}	8.58^{d}	8.61^{c}	8.68^{c}	8.85^{e}	17.26^{a}	14.16b^{a}
LSD ($P \leq 0.05$)		0.40	0.42	0.46	0.34	0.61	0.32	0.70	0.58

Values are means (n=3). Mean values in each citrus cultivar followed by different lower-case letters were significantly different at $P \leq 0.05$ by Tukey's HSD test.

to peak in February and declined thereafter. Overall the level of FAA ≥ 10 mg g^{-1} FW proved to be the critical level to induce flowering in all the genotypes of citrus tested during the course of present study. Recently, it was shown that the Asparagine (Asn) biosynthetic pathway also is active in flowering. Indeed, *ASPARAGINE SYNTHETASE1* (*ASN1*), encoding for the enzyme that transfers amide N from glutamine (Glu) to Asn, releasing Asn and Glu, display a high level of expression in flowers (Le *et al*., 11). Thus, enhancing the supply of Asn during flower development is an effective strategy to store reserves that will be used to generate energy during ovule maturation and embryo growth. The appreciable increment in the concentration of free amino acids in the shoots has been reported to the cause of attainment of ripeness to flower stage.

The total anti-oxidant capacity (TAC) was significantly affected by the genotypes at various dates of sampling. The TAC in Hill lemon and Mosambi and Pusa Round sweet oranges remained static till November, increased gradually till January and sharply till February, and declined thereafter (Table 7). Konkan Seedless lemon and Pusa Abhinav and Pusa Udit limes showed the sharp increase in TAC till September, then it became static till January, and then reached to a peak in February in Konkan Seedless lemon and Pusa Udit lime, however a sharp decline in Pusa Abhinav lime was recorded in October. The level of TAC ≥ 18.12 µmol g^{-1} FW favoured the flowering in all the genotypes of citrus listed during the course of present study. The TAC has been reported to be synthesized primarily in

Table 7. Seasonal variation in the total antioxidant capacity (TAC) (µmol g^{-1} FW) of citrus genotypes.

Species	Genotype	Month							
		Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar
C. aurantifolia	Pusa Udit	9.03^{e}	18.18^{d}	19.23^{b}	20.26^{a}	20.78^{a}	21.66^{a}	22.45^{g}	15.23^{e}
	Pusa Abhinav	9.68^{d}	19.15^{c}	12.17^{c}	20.19^{a}	20.33^{a}	20.69^{b}	24.40^{f}	15.56^{dec}
	ALC-29	20.05^{a}	20.10^{ba}	20.25^{a}	20.42^{a}	20.80^{a}	19.51^{c}	23.58^{f}	15.34^{de}
C. limon	Konkan Seedless	10.12^{d}	20.21^{a}	20.29^{a}	20.37^{a}	20.83^{a}	21.56^{a}	27.49^{d}	16.45^{a}
	Kagzi Kalan	18.12^{b}	19.27^{bc}	19.31^{b}	20.48^{a}	20.12^{a}	21.50^{ba}	29.44^{c}	16.2^{ba}
	Hill Lemon	10.90^{c}	9.21^{e}	9.88^{d}	10.34^{c}	14.52^{b}	15.56^{e}	28.47^{dc}	16.32^{ba}
C. sinensis	Pusa Sharad	9.05^{e}	9.18^{e}	10.25^{d}	10.28^{c}	14.78^{b}	16.45^{d}	31.67^{b}	15.89^{bdac}
	Pusa Round	9.08^{e}	9.45^{e}	10.01^{d}	10.18^{c}	15.12^{b}	15.90^{ed}	25.71^{e}	15.78^{bdec}
	Mosambi	9.78^{d}	10.02^{e}	10.11^{d}	12.25^{b}	15.08^{b}	16.12^{ed}	33.65^{a}	16.03^{bac}
LSD ($P \leq 0.05$)		0.60	0.89	0.67	0.58	0.87	0.83	1.04	0.64

Values are means (n=3). Mean values in each citrus cultivar followed by different lower-case letters were significantly different at $P \leq 0.05$ by Tukey's HSD test.

Table 8. Seasonal variation in the level of hydrogen peroxide (H_2O_2) (mmol H_2O_2 mg^{-1} FW) of citrus genotypes.

Species	Genotype	Month							
		Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar
C. aurantifolia	Pusa Udit	118.21^{ba}	101.30^{c}	105.63^{b}	101.48^{b}	105.63^{b}	107.75^{b}	102.71^{bcd}	101.12^{bac}
	Pusa Abhinav	120.65^{ba}	105.99^{cb}	121.09^{a}	104.30^{b}	108.63^{b}	110.40^{b}	103.22^{bc}	102.21^{ba}
	ALC-29	102.45^{c}	102.64^{cb}	101.91^{b}	102.97^{b}	106.52^{b}	108.21^{b}	101.97^{bcd}	99.25^{bc}
C. limon	Konkan Seedless	116.49^{ba}	107.49^{b}	106.16^{b}	105.44^{b}	106.38^{b}	108.29^{b}	98.89^{d}	96.45^{c}
	Kagzi Kalan	105.34^{c}	107.34^{b}	108.45^{b}	106.63^{b}	107.82^{b}	109.45^{b}	100.52^{cd}	99.89^{bc}
	Hill Lemon	120.75^{ba}	121.75^{a}	123.00^{a}	129.53^{a}	136.42^{a}	145.46^{a}	105.72^{ba}	101.89^{ba}
C. sinensis	Pusa Sharad	121.36^{a}	122.07^{a}	124.01^{a}	127.24^{a}	135.19^{a}	142.27^{a}	107.98^{a}	105.45^{a}
	Pusa Round	119.29^{ba}	121.29^{a}	123.12^{a}	128.83^{a}	136.18^{a}	140.65^{a}	103.25^{bc}	101.47^{ba}
	Mosambi	116.02^{b}	119.02^{a}	122.70^{a}	126.93^{a}	133.58^{a}	141.21^{a}	108.27^{a}	105.56^{a}
LSD ($P \leq 0.05$)		4.89	5.73	7.23	6.47	4.80	5.83	4.14	4.80

Values are means (n=3). Mean values in each citrus cultivar followed by different lower-case letters were significantly different at $P \leq 0.05$ by Tukey's HSD test.

the plants for own defence against the oxidative stress. The composition of bioactive components and antioxidant capacity in the plant were significantly affected by the developmental stages (Brown *et al*., 2).

Various citrus genotypes differed significantly in respect of the level of peroxide (H_2O_2) studied at monthly intervals (Table 8). In the day neutral responsive citrus genotypes (all limes and Konkan Seedless and Kagzi Kalan lemons), the level of H_2O_2 remained static, which declined marginally after January month. However, sweet oranges and Hill lemon, its level increased sharply (140.65-145.46 mmol g^{-1} FW) in January and decline thereafter. Overall low level of H_2O_2 favoured the flowering in all the genotypes of citrus studied during the course of present study. Several studies have indicated that controlled production of ROS is vital for cell differentiation and expansion during the morphogenesis of organs (Zinta *et al*., 20). The studies of Lokhande *et al*. (12) further supported our contention that production of ROS like H_2O_2 was important to flower induction. However, the increase in the antioxidant enzymes might be the possible cause of sudden decline in the levels of ROS, as has also been observed in the present study.

Of the various citrus genotypes, ALC-29 lime and Kagzi Kalan lemon behaved as everbearer throughout the period of sampling. The photosynthetic rate (*A*) remained high in non-flowering state. Carbohydrate, C:N ratio and FAA increased towards the approach spring flowering. In sweet orange and Hill lemon, GA_3 followed an upward trend till January, and declined thereafter. In rest of the genotypes, the reverse trend was noticed till December. Antioxidants activity (TAC) increased till February, while ROS (H_2O_2) increased till January and declined thereafter.

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Received : August, 2019; Revised : February, 2020;
Accepted : March, 2020